Urological Science 23 (2012) 107–113

Contents lists available at SciVerse ScienceDirect

Urological Science

journal homepage: www.urol-sci.com

Original article

Protein expression profiling in interstitial cystitis/painful bladder syndrome: A pilot study of proteins associated with inflammation, apoptosis, and angiogenesis

Jia-Heng Shie [a,b], Hsin-Tzu Liu [a,b], Hann-Chorng Kuo [a,b,*]

[a] Department of Urology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[b] Department of Urology, Tzu Chi University, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 6 December 2011
Received in revised form
6 January 2012
Accepted 5 March 2012
Available online 5 December 2012

Keywords:
angiogenesis
apoptosis
inflammation
interstitial cystitis
painful bladder syndrome

ABSTRACT

Objective: Protein arrays are rapidly becoming a powerful means to detect proteins, monitor their expression levels, and investigate protein interactions and functions. We used this technology and demonstrated its application in identifying potential biomarkers for the diagnosis and treatment of interstitial cystitis/painful bladder syndrome (IC/PBS).
Materials and Methods: To compare the expression profiles of 40 proteins related to inflammation, 30 proteins related to apoptosis, and 20 proteins related to angiogenesis, we performed a protein array assay using bladder tissues of three normal and six IC/PBS patients and urine samples at the baseline and after three repeated intravesical onabotulinumtoxinA injections. Different protein expression levels were determined using the Image J process. After the onabotulinumtoxinA injections, urine samples of IC/PBS the patient were analyzed using angiogenesis proteins, and results were compared with the untreated baseline data.
Results: Of all inflammatory antigens, 15 were slightly promoted in the IC/PBS patients' bladder tissues. Moreover, 80% of proapoptotic proteins and 10% of antiapoptotic proteins on the protein array increased. We also found that several angiogenesis cytokines decreased in the urine of the IC/PBS patients after the onabotulinumtoxinA injections.
Conclusion: Our results indicate that several signal transduction pathways are involved in the pathophysiology of IC/PBS and provide valuable information on the signal networks of different pathways in IC/PBS, including inflammation, apoptosis, and angiogenesis. This is a powerful database to further investigate molecular mechanisms of IC/PBS.

1. Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a chronic, painful condition of the bladder wall characterized by pressure and pain above the pubic area along with increased frequency of urination and glomerulations in cystoscopic hydrodistention. In patients with IC/PBS, researchers have found specific pathologies, including abnormalities of the urothelium, changes in the nerve function within the bladder wall, and mast cell overactivation.[1,2] This evidence suggests that several possible pathogenetic developmental pathways are involved in IC/PBS, including chronic inflammation.[3] Histologic studies of tissue biopsies of IC/PBS patients have consistently reported signs of inflammation as evidenced by degranulation of mast cells and infiltration of mast cells, macrophages, and neutrophils.[4]

One of the most common findings in IC/PBS patients is denudation or thinning of the bladder epithelium, suggesting altered regulation of urothelial homeostasis.[1] Our previous reports indicated that the terminal deoxynucleotidyl transferase nick end labeling (TUNEL)-positive cells were present in the bladder of IC/PBS patients, and there was a significant positive correlation between mast cell activation and the apoptotic cell number in bladder tissues of patients with IC/PBS.[5] These results suggest that the urothelium plays a pivotal role as a barrier between the urine (and its solutes) and the underlying bladder tissues.

During an inflammatory response, leukocytes must leave the vascular lumen and enter the tissue where they perform effecter functions. Signaling molecules and cytokines such as tumor necrosis factor (TNF) and interleukin (IL)-1 cause the expressions of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 by neutrophils, which trigger vascular permeability abnormalities or physiologic angiogenesis.[6] The cardinal symptoms and signs of IC/PBS, such as bladder pain, increased bladder sensation, diffused glomerulations during hydrodistention,

* Corresponding author. Department of Urology, Buddhist Tzu Chi General Hospital, 707 Chung-Yang Road, Section 3, Hualien 970, Taiwan.
E-mail address: hck@tzuchi.com.tw (H.-C. Kuo).

1879-5226
http://dx.doi.org/10.1016/j.urols.2012.10.003

and reduced bladder capacity, all reflect the inflammatory process in the diseased bladder wall, and neural upregulation occurs both peripherally and centrally in the bladders of patients with IC/PBS.[7]

The clinical presentations of IC/PBS are heterogeneous.[2] Patients with IC/PBS may have predominant bladder pain or predominant urgency frequency symptoms. Under cystoscopic hydrodistention, some patients may have a reduced maximum bladder capacity under anesthesia or mild to severe glomerulations or ulceration, but some do not show these features.[8] Although urine and serum biomarkers such as nerve growth factor (NGF), C-reactive protein (CRP), anti-proliferative factor (APF), heparin-binding epidermal growth factor-like growth factor (HB-EGF), and epidermal growth factor (EGF) were found to be elevated in IC/PBS[1,9–11] not all IC/PBS bladders have elevated urinary biomarker levels, although they have typical bladder pain symptoms and cystoscopic findings.[3,9,10] Increased bladder mast cell activity[9] and proinflammatory and apoptotic protein expressions are also not consistently found in all IC/PBS bladder biopsies.[5,12] Recent studies also revealed that the mast-cell density does not appear to correlate with the duration of symptom amelioration after complete transurethral resection of Hunner ulcer,[13] and overall urine markers were not associated with biopsy findings.[14] Therefore, we speculated that histologic findings, urinary biomarkers, and clinical presentations in IC/PBS might be heterogeneous.

In this study, we present the preliminary results of a proteomics analysis of the processes of inflammation, angiogenesis, and apoptosis in IC/PBS bladder tissues and urine samples, and focused on the crosstalk between different signaling pathways. Based on the proteomics findings in IC/PBS bladders, it is possible to link chronic inflammation, urothelial apoptosis, and angiogenesis in IC/PBS. The results may facilitate future investigations and provide evidence for therapy targeting chronic inflammation in IC/PBS patients.

2. Patients and methods

Six women with characteristic symptoms of IC/PBS and diffuse glomerulations but no Hunner ulcer on cystoscopic hydrodistention were enrolled in this study. Another three women with stress urinary incontinence without bladder symptoms served as the controls. Bladder biopsies at three sites were taken immediately after cystoscopic hydrodistention for the diagnosis of IC/PBS and from normal controls during anti-incontinence procedures. No patient had previously been treated for IC/PBS before the bladder procedure. Urine samples were also obtained from one patient with Grade 2 glomerulations at the baseline who received three repeated intravesical onabotulinumtoxinA (BoNT-A) injections, and follow-up cystoscopic hydrodistention revealed completely resolution of the glomerulations.

This study was approved by the Institutional Review Board and Ethics Committee of Buddhist Tzu Chi General Hospital. Each patient was informed about the study rationale and procedures, and written informed consent to participate in the study was obtained before we began the bladder procedures.

The IC/PBS patients were classified by the degree of glomerulations after hydrodistention as Grades 0–3, respectively representing none, mild, moderate, and severe IC/PBS.[2] Specimens with Grade 2 and 3 (G2 or G3) for glomerulations without an ulcer were used in this study.

The patients underwent three bladder biopsies at about 2 cm above the ureteral orifice on the lateral and posterior walls. Each specimen was 2 mm in diameter, and only bladder mucosa was obtained. The bladder biopsy specimens were sent to the pathology department for hematoxylin and eosin staining to exclude the possibility of carcinoma in situ. Specimens were also stored frozen in liquid nitrogen for further investigations. The bladder biopsies were taken at the same sites in the controls and were prepared using the same methods for comparison.

To compare the expression profiles of 30 proteins related to inflammation and 35 proteins related to apoptosis, we performed a protein array assay on the bladder tissues of normal and IC/PBS patients and identified several differentially expressed proteins using the Image J software (http://rsb.info.nih.gov/ij/index.html).[15] After the three BoNT-A injections, urine samples of the IC/PBS patient were analyzed for 20 angiogenesis proteins, and the results were compared with untreated baseline data.

2.1. Human apoptosis protein and cytokine antibody array

Extracted protein lysates (250 mg) of control and IC/PBS specimens were incubated on a human apoptosis protein array membrane (R&D Systems, Minneapolis, MN, USA) at 4°C overnight, washed three times for 5 minutes with a wash buffer, and then incubated with a horseradish peroxidase (HRP)-linked secondary antibody diluted 1:2000. After washing, blotted dots were visualized using a Western chemiluminescent HRP substrate (Millipore, Guyancourt, France). Normalized intensities were calculated from each array by subtracting the local background from each spot, and the data were corrected for the protein content in each well. Then multiples of change in protein expression levels between IC/PBS and control specimens were calculated.

The human cytokine antibody array procedure (RayBiotech, Norcross, GA, USA) was conducted according to the manufacturer's instructions. Briefly, membranes were blocked by incubation with blocking buffer at room temperature for 90 minutes. Membranes were washed three times with wash buffer I and two times with wash buffer II at room temperature for 5 minutes per wash, and incubated with biotin-conjugated antibodies at room temperature for 90 minutes. Finally, membranes were washed and incubated with HRP-conjugated streptavidin at room temperature for 2 hours. The chemiluminescent protein dot signals were measured as described above.

To investigate changes in angiogenesis proteins in IC/PBS bladders after three repeated BoNT-A injections, the urine of one IC/PBS patient was analyzed using an angiogenesis protein array, and the results were compared with the untreated baseline of the same patient. The patient exhibited improvement of glomerulations from Grade 2 to Grade 0 after BoNT-A treatment (Fig. 1A). Two urine samples were used for the protein array, and 20 molecules for angiogenesis and vascular inflammation were investigated. The multiples of change in protein expression levels between the baseline and post-treatment in urine samples of IC/PBS patient were calculated. The results of the angiogenesis protein array from urine samples at the baseline and post-treatment might provide evidence of the effect of intravesical BoNT-A treatment.

2.2. Statistical analysis

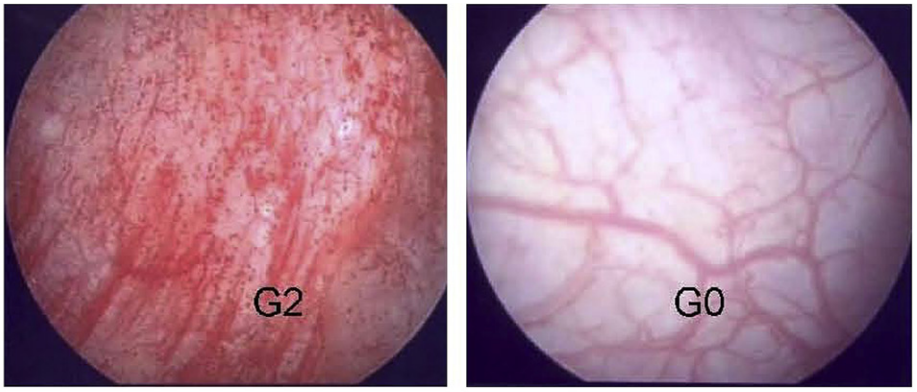
The intensities of proteins in the arrays and Western blots were quantified using Image J processing.[15] Differences in expressions of inflammatory and apoptotic proteins between IC/PBS and control specimens were analyzed using the Kruskal-Wallis test. All calculations were performed using SPSS for Windows, version 10.0 (SPSS, Chicago, IL, USA). A p value of <0.05 was considered statistically significant.

3. Results

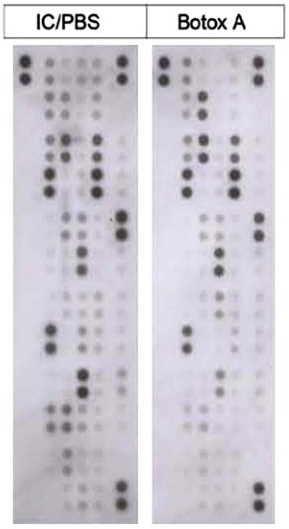
3.1. Inflammatory proteins

The inflammatory protein array comprised 30 spots of inflammation-related molecules spotted in duplicate, and positive

A



B



psotive control	serpin B5	MIP-1α	GDNF	coagulation factor III	psotive control
psotive control	serpin B5	MIP-1α	GDNF	coagulation factor III	psotive control
	serpin E1	MMP-8	GM-SCF	CXCL16	
	serpin E1	MMP-8	GM-SCF	CXCL16	
	serpin F1	MMP-9	HB-EGF	DPPIV	activin A
	serpin F1	MMP-9	HB-EGF	DPPIV	activin A
	TIMP-1	NRG1-β1	HGF	EGF	ADAMTS-1
	TIMP-1	NRG1-β1	HGF	EGF	ADAMTS-1
	TIMP-4	Pentraxin 3	IGFBP-1	EG-VEGF	angiogenin
	TIMP-4	Pentraxin 3	IGFBP-1	EG-VEGF	angiogenin
	Thrombospondin-1	PD-ECGF	IGFBP-2	Endoglin	angiopoietin-1
	Thrombospondin-1	PD-ECGF	IGFBP-2	Endoglin	angiopoietin-1
	Thrombospondin-2	PDGF-AA	IGFBP-3	Endostatin	angiopoietin-2
	Thrombospondin-2	PDGF-AA	IGFBP-3	Endostatin	angiopoietin-2
	Upa	PDGF-AB/-BB	IL-1β	Endithelin-1	agiostatin
	Upa	PDGF-AB/-BB	IL-1β	Endithelin-1	agiostatin
	vasohibin	Persephin	IL-8	FGF acidic	amphiregulin
	vasohibin	Persephin	IL-8	FGF acidic	amphiregulin
	VEGF	platelet factor 4	TGF-β1	FGF basic	artemin
	VEGF	platelet factor 4	TGF-β1	FGF basic	artemin
	VEGF-C	PlGF	Leptin	FGF-4	
	VEGF-C	PlGF	Leptin	FGF-4	
negativee control		Prolactin	MCP-1	FGF-7	psotive control
negativee control		Prolactin	MCP-1	FGF-7	psotive control

C

	spot pixel mean value		
Protein	IC/PBS base line	Botox A injected	Fold
TIMP-4	45.119	32.194	0.713
VEGF	79.342	51.158	0.644
VEGF-C	68.951	49.105	0.712
Pentraxin 3	65.06	44.857	0.689
platelet factor 4	86.469	54.087	0.625
PlGF	83.057	56.943	0.685
GDNF	73.891	50.620	0.685
GM-SCF	57.729	41.825	0.724
IL-1β	59.344	40.261	0.678
IL-8	106.74	64.138	0.600
TGF-β1	65.570	44.168	0.673
coagulation factor III	72.794	54.375	0.746
CXCL16	62.271	39.836	0.639
amphiregulin	62.811	46.584	0.741

Fig. 1. Angiogenesis protein array in an interstitial cystitis/painful bladder syndrome (IC/PBS) patient at baseline and after intravesical onabotulinumtoxinA (BoNT-A) treatment. Results were compared with the untreated baseline of the same patient. Multiples of changes in protein expression levels between baseline and post-treatment in urine samples of the IC/PBS patient were calculated. (A) Glomerulations at baseline were Grade 2 and became Grade 0 after treatment; (B) among angiogenesis- and vascular inflammatory-related proteins in the array membrane, 92% were suppressed; (C) protein expressions dramatically decreased after BoNT-A injections, such as vascular endothelial growth factor, platelet factor 4, interleukin (IL)-1β, IL-8, chemokine (C-X-C motif) ligand 16, and tissue inhibitor of metalloproteinase-4.

and negative controls were fabricated as described in the Methods section. We measured the results using Image J software and identified molecular changes in inflammatory signals. Among the 30 proteins in the array, 14 proteins slightly increased in bladder tissue lysates of IC/PBS samples compared with the controls, including IL-10, IL-11, and IL-12; TNF-α signal-related molecules (sTNFR-I and II), macrophage inflammatory proteins (MIPs-1α, β, and δ); fibrosis-associated tissue inhibitors of metalloproteinase (TIMP)-2; and intercellular adhesion molecule (ICAM)-1 (Fig. 2).

3.2. Apoptotic proteins

To better characterize molecular events leading to apoptosis in IC/PBS samples, we conducted an apoptosis-related protein-antibody array analysis of control and IC/PBS specimens. Results showed that 15 pro- or antiapoptotic proteins were significantly modulated (Fig. 3). Among proapoptotic proteins known to mediate the apoptotic process, levels of Bax, TRAIL R2, FADD, Fas, and phospho-p53 increased. Strikingly, some antiapoptotic proteins, such as Bcl-2, members of the heat shock proteins (HSPs), cyclin-dependent kinase (CDK) inhibitor, p21, and p27, also significantly increased.

3.3. Angiogenesis proteins

After BoNT-A treatment, the urine of the IC/PBS patient was analyzed using an angiogenesis protein array, and results were compared with the untreated baseline of the same patient. As shown in Fig. 1B, 92% of molecules related to angiogenesis and vascular inflammation on the array membrane were suppressed. Fig. 1C shows that protein expressions dramatically decreased after the BoNT-A injections, such as vascular endothelial growth factor (VEGF), platelet factor 4, IL-1β, IL-8, chemokine (C-X-C motif) ligand 16, and TIMP-4.

4. Discussion

This was a pilot study investigating protein expression profiles of inflammation, urothelial apoptosis, and angiogenesis in IC/PBS patients using a protein array technique. Further confirmative studies are mandatory in order to explore the heterogeneity of IC/PBS and mechanisms of therapeutic effects of varying treatment modalities for IC/PBS.

Previous studies demonstrated that IC/PBS patients have altered bladder epithelial expressions of HLA-DR and ICAM-a, and secreted proteins, including IL-1, TNF-α, and various epithelial growth factors such as HB-EGF, EGF, and IGF1.[16–19] Studies on urothelial dysfunction in IC/PBS also showed altered expressions of certain tight junction proteins such as zona occludens-1, occluding, and claudins 1, 4, and 8.[20,21] Urine samples from IC/PBS patients also showed significantly decreased HB-EGF and increased levels of EGF, IGF1, IGFBP3, and cytokines, including IL-6 and IL-8.[22] Those findings indicate that several inflammatory processes are involved in IC/PBS and are responsible for the clinical symptoms and signs.

Basic research has demonstrated that cardinal signs of inflammation such as infiltration of mononuclear cells, activation of mast cells, and neovascularization (angiogenesis) are orchestrated by chemokines, cytokines, and growth factors in IC/PBS bladders.[23,24] During the development of inflammation, two major events occur. Leukocytes interact with the endothelium in a process that allows these cells to cross the barrier created by endothelial cells. This cellular migration results in modifications of the vascular permeability that permits the transfer of solutes to peripheral tissues. Under basal conditions, alterations in vascular permeability are linked to ICAM-1 expression in a protein kinase C (PKC)-dependent manner.[25]

Matrix metalloproteinases (MMPs) can degrade components of the extracellular matrix (ECM). Activities of MMPs are inhibited by tissue inhibitors of metalloproteinases (TIMPs) through formation of non-covalent 1:1 complexes with MMPs. Four members of the TIMP family have been characterized so far, designated TIMP-1, -2, -3, and -4. TIMP-1 is capable of inhibiting the activities of most MMPs, and plays a key role in maintaining the balance between ECM deposition and degradation as several inflammatory mediators are substrates of MMPs. TIMP-1 might affect renal fibrosis via an inflammatory pathway.[26]

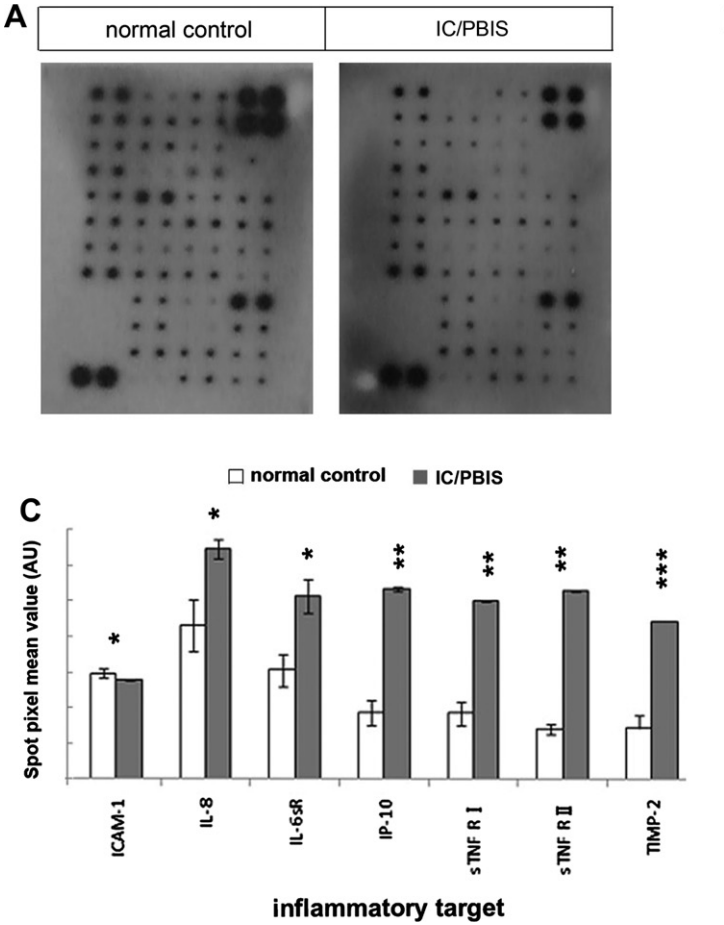
A multitude of vasoactive cytokines, growth factors, and signal modulators react with endothelial cell substructure components to control permeability. VEGF, IL-α and -β, TNF-α, and interferon (IFN)-γ were shown to increase endothelial monolayer permeability.[27] Lipopolysaccharide (LPS) induces junction barrier loss and cell detachment by activating PTKs and caspase cleavage reactions.[28] By contrast, junctional adhesion molecules (JAMs) decrease permeability by initiating cell adhesion. Endothelial cell adhesive characteristics provide strength and stability for neighboring cells and the cellular cytoskeleton by interacting with actin and myosin contractile filaments.[29] Thrombin stimulation of cytoskeletal signaling pathways was shown to manipulate cell permeability. Junctional molecules also influence cell signaling and trigger responses that are translated into cell morphological changes and physiological angiogenesis.[30]

Incubation of human aortic endothelial cells (HAECs) with IL-1α resulted in increased permeability of quercetin-3-glucuronide. Furthermore, quercetin-3-glucuronide showed no suppressive effect on TNF-α-induced cell expression of ICAM-1 by HAECs. MIP-1α is a member of the C-C subfamily of chemokines, a large superfamily of low-molecular-weight, inducible proteins that exhibit a variety of proinflammatory activities *in vitro*, including leukocyte chemotaxis.[31]

Among apoptotic and inflammatory proteins, Bad, Bax, caspase 3, p53, p27, and TNF-α were confirmed by Western blot and statistical analyses to have higher expression levels in IC/PBS bladders than in normal bladders.[12] In this pilot study, changes in Bad and caspase 3 did not reach statistical significance, possibly due to the small sample size. Previous research also indicated that phosphorylation of p53 by p38α can play a role in p53-dependent transcription.[32] In addition, p53 is translocated into mitochondria to interact with Bcl-2 or Bcl-xl, thereby activating Bax directly or indirectly, and leading to caspase-dependent apoptosis. Previously, evidence showed that the extranuclear role of p53 in inducing apoptosis is through mechanisms involving Bad and Bax, which are the main factors that mediate mitochondrial dysfunction and cell apoptosis.[33] Additionally, p53 also participates in cell cycle arrest through inducing p21 and p27 activities. These proteins are potential biomarkers for diagnosing IC/PBS. Our findings indicate that apoptosis and growth arrest of bladder tissues of IC/PBS patients could be due to upregulation of inflammatory signals.

In addition, TNF-α also promotes apoptosis through binding to TNF-receptor 1, resulting in many effects ranging from inflammation to apoptosis.[34] TNF-α is a pleiotropic molecule that plays a central role in inflammation, apoptosis, and immune system development.[35] Previously, the nature of neurogenic pain was studied in animal models, and these pain models indicated that cytokines (TNF-α and NGF) were upregulated in injured nerves, and neutralizing TNF-α reduced pain behavior.[36,37] More exactly, the primary role of TNF-α is in regulating immune cells, and it can also induce apoptotic cell death through activation of the transcription factors, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun N-terminal kinase.[38]

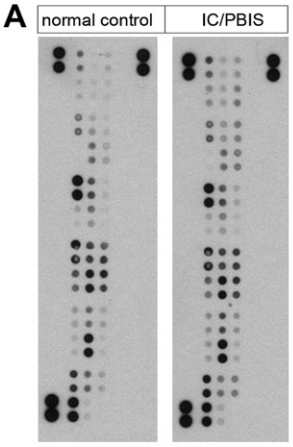
Taken together, our protein array results revealed that several inflammatory proteins were significantly increased in bladder tissues of IC/BPS patients, and several proapoptotic and antiapoptotic proteins increased. The urine angiogenesis array also showed decreases in angiogenesis and inflammatory proteins after



B

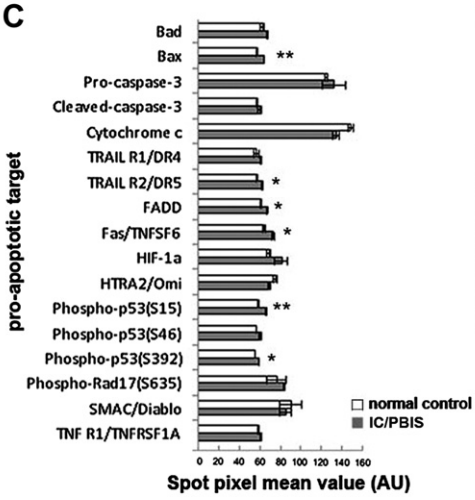
RANTES	RANTES	IL-13	IL-13	IL-1β	IL-1β	positive con	positive con
TGF-β1	TGF-β1	IL-15	IL-15	IL-2	IL-2	Positive con	positive con
TGF-α	TGF-α	IL-16	IL-16	IL-3	IL-3	negative con	negative con
TGF-β	TGF-β	IL-17	IL-17	IL-4	IL-4	negative con	negative con
sTNFR I	sTNFR I	IP-10	IP-10	IL-6	IL-6	EOTAXIN	EOTAXIN
sTNFR II	sTNFR II	MCP-1	MCP-1	IL-6sR	IL-6sR	EOTAXIN-2	EOTAXIN-2
PDGF-BB	PDGF-BB	MCP-2	MCP-2	IL-7	IL-7	GCSF	GCSF
TIMP-2	TIMP-2	M-CSF	M-CSF	IL-8	IL-8	GM-GCSF	GM-GCSF
BLANK	BLANK	MIG	MIG	IL-10	IL-10	ICAM-1	ICAM-1
BLANK	BLANK	MIP-1α	MIP-1α	IL-11	IL-11	IFN-γ	IFN-γ
negative con	negative con	MIP-1β	MIP-1β	IL-12p40	IL-12p40	I-309	I-309
positive con	positive con	MIP-1δ	MIP-1δ	IL-12p70	IL-12p70	IL-1α	IL-1α

Fig. 2. (A) Bladder tissue lysates of control and interstitial cystitis/painful bladder syndrome (IC/PBS) specimens were applied to a human inflammatory antibody array; (B) spots of each protein refer to the table for the array coordinates; (C) average density of duplicate spots representing each inflammatory protein is expressed in arbitrary units. Seven proteins significantly increased in the IC/PBS samples compared with the controls, including intercellular adhesion molecule, interleukin (IL)-8, IL-6sR, inflammatory protein-10, tumor necrosis factor (TNF)-α signal-related molecule (sTFN-R I), sTFN-R II, and tissue inhibitor of metalloproteinase-2. The significance of changes in protein expression levels between the control and IC/PBS samples was determined using the Kruskal-Wallis test. $^{*}p < 0.05$. $^{**}p < 0.005$. $^{***}p < 0.001$.



B

positive con	Bad	TRAIL R1/DR4	PON2	positive con
positive con	Bad	TRAIL R1/DR4	PON2	positive con
	Bax	TRAIL R2/DR5	p21/CIP1/CDNK1A	
	Bax	TRAIL R2/DR5	p21/CIP1/CDNK1A	
	Bcl-2	FADD	p27/Kip1	
	Bcl-2	FADD	p27/Kip1	
	Bcl-x	Fas/TNFSF6	Phospho-p53(S15)	
	Bcl-x	Fas/TNFSF6	Phospho-p53(S15)	
	Pro-caspase-3	HIF-1a	Phospho-p53(S46)	
	Pro-caspase-3	HIF-1a	Phospho-p53(S46)	
	Cleaved-caspase-3	HO-1/HMOX1/HSP32	Phospho-p53(S392)	
	Cleaved-caspase-3	HO-1/HMOX1/HSP32	Phospho-p53(S392)	
	Catalase	H0-2/HMOX2	Phospho-Rad17(S635)	
	Catalase	H0-2/HMOX2	Phospho-Rad17(S635)	
	cIAP-1	HSP27	SMAC/Diablo	
	cIAP-1	HSP27	SMAC/Diablo	
	cIAP-2	HSP60	Survivin	
	cIAP-2	HSP60	Survivin	
	Claspin	HSP70	TNF R1/TNFRSF1A	
	Claspin	HSP70	TNF R1/TNFRSF1A	
	Clusterin	HTRA2/Omi	XIAP	
	Clusterin	HTRA2/Omi	XIAP	
positive con	Cytochrome c	Livin	PBS(- con)	
positive con	Cytochrome c	Livin	PBS(- con)	



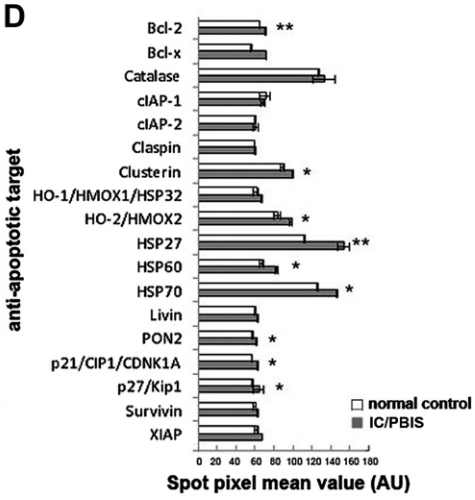


Fig. 3. (A) Bladder tissue lysates of control and interstitial cystitis/painful bladder syndrome (IC/PBS) specimens applied to a human apoptosis protein array. Results are shown after exposure of the array membranes to X-ray film for 1 minute; (B) comprehensive protein membrane-array map. The human protein array simultaneously profiles 35 proteins in duplicate, a set of six positive controls and two negative controls. The average density of duplicate spots representing each pro- and antiapoptotic protein is expressed in arbitrary units; (C, D) several molecules significantly increased in the tissue lysate of the IC/PBS samples, including Bax, TRAIL R2, FADD, Fas, and phospho-p53 (Ser15) in proapoptotic targets, and Bcl-2, clusterin, H0-2, heat shock protein (HSP) 27, HSP60, HSP70, PON2, p21, and p27 in antiapoptotic targets. The significance of changes in protein expression between control and IC/PBS specimens was determined using the Kruskal-Wallis test. $^{*}p < 0.05$. $^{**}p < 0.005$.

three repeated intravesical BoNTA injections. These protein expression profiles of inflammation, apoptosis, and angiogenesis suggest a network might exist in the IC/PBS bladder and could explain the pathophysiology of the clinical features of IC/PBS. Although this is a small pilot study, the results can provide valuable evidence for future investigations of heterogeneous pathogenesis and therapeutic effects of treatments for IC/PBS.

The limitation of this study is the small sample number (six IC/PBS patients and three controls), and only the bladder mucosa was harvested. However, because this is a pilot study for the protein profile study of IC/PBS patients, results of this study can help us select appropriate proteins for further large-scale investigations of the pathophysiology and treatment results of IC/PBS.

5. Conclusions

In this study, we describe the first findings obtained by proteomic studies of the molecular mechanisms of IC/PBS, including correlations among inflammation, angiogenesis, and apoptosis. To further confirm the information obtained from the protein array, advanced analytical techniques such immunoblotting and enzyme-linked immunosorbent assay are required. We provide a powerful research protocol for understanding the pathology of IC/PBS, and even other urologic diseases.

Conflicts of interest statement

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

Source of Funding

None.

References

1. Keay S. Cell signaling in interstitial cystitis/painful bladder syndrome. *Cell Signal* 2008;**20**:2174–9.
2. Homma Y, Ueda T, Tomoe H, Tomoe H, Lin AT, Kuo HC, et al. Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome. *Int J Urol* 2009;**16**: 597–615.
3. Sant GR, Kempuraj D, Marchand JE, Theoharides TC. The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis. *Urology* 2007;**69**:34–40.
4. Erickson DR, Belchis DA, Dabbs DJ. Inflammatory cell types and clinical features of interstitial cystitis. *J Urol* 1997;**158**:790–3.

5. Shie JH, Kuo HC. Higher levels of cell apoptosis and abnormal E-cadherin expression in the urothelium are associated with inflammation in patients with interstitial cystitis/painful bladder syndrome. *BJU Int* 2011;**108**:E136–41.
6. Weber C, Fraemohs L, Dejana E. The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* 2007;**7**:467–77.
7. Nazif O, Teichman JM, Gebhart GF. Neural upregulation in interstitial cystitis. *Urology* 2007;**69**(Suppl):24–33.
8. Bouchelouche K, Nordling J. Recent developments in the management of interstitial cystitis. *Curr Opin Urol* 2003;**13**:309–13.
9. Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor level is increased in patients with interstitial cystitis/bladder pain syndrome and decreased in responders to treatment. *BJU Int* 2009;**104**:1476–81.
10. Liu HT, Tyagi P, Chancellor MB, Kuo HC. Urinary nerve growth factor but not prostaglandin E2 increases in patients with interstitial cystitis/bladder pain syndrome and detrusor overactivity. *BJU Int* 2010;**106**:1681–5.
11. Chung SD, Liu HT, Lin H, Kuo HC. Elevation of serum c-reactive protein in patients with OAB and IC/BPS implies chronic inflammation in the urinary bladder. *Neurourol Urodyn* 2011;**30**:417–20.
12. Shie JH, Liu HT, Kuo HC. Increased cell apoptosis of the urothelium is closely linked to chronic inflammation in interstitial cystitis/painful bladder syndrome. *Urology* 2012;**79**:484.e7–13.
13. Rossberger J, Fall M, Gustafsson CK, Peeker R. Does mast cell density predict the outcome after transurethral resection of Hunner's ulcer lesions in patients with type 3C bladder pain syndrome/interstitial cystitis? *Scand J Urol Nephrol* 2010;**44**:433–7.
14. Erickson DR, Tomzazewski JE, Kunselman AR, Stetter CM, Peters KM, Rovner ES, et al. Urine maerkers do not predict biopsy findings or presence of bladder ulcers in interstitial cystitis/painful bladder syndrome. *J Urol* 2008;**179**:1850–6.
15. Kaczmarek E, Gorna A, Majewski P. Techniques of image analysis for quantitative immunohistochemistry. *Rocz Akad Med Bialymst* 2004;**49** (Suppl. 1):155–8.
16. Liebert M, Wedemeyer G, Stein JA, Washington Jr R, Faerber G, Flint A, et al. Evidence for urothelial cell activation in interstitial cystitis. *J Urol* 1993;**149**: 470–5.
17. Christmas TJ, Bottazzo GF. Abnormal urothelial HLA-DR expression in interstitial cystitis. *Clin Exp Immunol* 1992;**87**:450–4.
18. Keay S, Kleinberg M, Zhang CO, Hise MK, Warren JW. Bladder epithelial cells from patients with interstitial cystitis produce an inhibitor of heparin-binding epidermal growth factor-like growth factor production. *J Urol* 2000;**164**:2112–8.
19. Zhang CO, Wang JY, Koch KR, Keay S. Regulation of tight junction proteins and bladder epithelial paracellular permeability by an antiproliferative factor from patients with interstitial cystitis. *J Urol* 2005;**174**:2382–7.
20. Keay S, Seillier-Moiseiwitsch F, Zhang CO, Chai TC, Zhang J. Changes in human bladder epithelial cell gene expression associated with interstitial cystitis or antiproliferative factor treatment. *Physiol Genomics* 2003;**14**:107–15.
21. Keay S, Zhang CO, Kagen DI, Hise MK, Jacobs SC, Hebel JR, et al. Concentrations of specific epithelial growth factors in the urine of interstitial cystitis patients and controls. *J Urol* 1997;**158**:1983–8.
22. Erickson DR, Xie SX, Bhavanandan VP, Wheeler MA, Hurst RE, Demers LM, et al. A comparison of multiple urine markers for interstitial cystitis. *J Urol* 2002;**167**: 2461–9.
23. Ueda T, Tamaki M, Ogawa O, Yoshimura N. Over expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in patients with interstitial cystitis and bladder carcinoma. *J Urol* 2002;**167**:347–51.
24. Parsons CL, Stein P, Zupkas P, Chenoweth M, Argade SP, Proctor JG, et al. Defective Tamm-Horsfall protein in patients with interstitial cystitis. *J Urol* 2007;**178**:2665–70.
25. Frank PG, Lisanti MP. ICAM-1: role in inflammation and in the regulation of vascular permeability. *Am J Physiol Heart Circ Physiol* 2008;**295**:H926–7.
26. Cai G, Zhang X, Hong Q, Shao F, Shang X, Fu B, et al. Tissue inhibitor of metalloproteinase-1 exacerbated renal interstitial fibrosis through enhancing inflammation. *Nephrol Dial Transplant* 2008;**23**:1861–75.
27. Lal BK, Varma S, Pappas PJ, Hobson 2nd RW, Duran WN. VEGF increases permeability of the endothelial cell monolayer by activation of PKB/akt, endothelial nitric-oxide synthase, and MAP kinase pathways. *Microvasc Res* 2001;**62**:252–62.
28. Bannerman DD, Sathyamoorthy M, Goldblum SE. Bacterial lipopolysaccharide disrupts endothelial monolayer integrity and survival signaling events through caspase cleavage of adherens junction proteins. *J Biol Chem* 1998; **273**:35371–80.
29. Dudek SM, Garcia JG. Cytoskeletal regulation of pulmonary vascular permeability. *J Appl Physiol* 2001;**91**:1487–500.
30. Krizbai IA, Deli MA. Signalling pathways regulating the tight junction permeability in the blood-brain barrier. *Cell Mol Biol (Noisy-le-grand)* 2003;**49**:23–31.
31. Cook DN. The role of MIP-1 alpha in inflammation and hematopoiesis. *J Leukoc Biol* 1996;**59**:61–6.
32. Ambrosino C, Nebreda AR. Cell cycle regulation by p38 MAP kinases. *Biol Cell* 2001;**93**:47–51.
33. Jiang P, Du W, Wu M. p53 and Bad: remote strangers become close friends. *Cell Res* 2007;**17**:283–5.
34. Rath PC, Aggarwal BB. TNF-induced signaling in apoptosis. *J Clin Immunol* 1999;**19**:350–64.
35. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microsc Res Tech* 2000;**50**:184–95.
36. Herzberg U, Eliav E, Dorsey JM, Gracely RH, Kopin IJ. NGF involvement in pain induced by chronic constriction injury of the rat sciatic nerve. *Neuroreport* 1997;**8**:1613–8.
37. Schafers M, Geis C, Brors D, Yaksh TL, Sommer C. Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve. *J Neurosci* 2002;**22**:536–45.
38. Gaur U, Aggarwal BB. Regulation of proliferation, survival and apoptosis by members of the TNF superfamily. *Biochem Pharmacol* 2003;**66**:1403–8.